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ELECTROKINETIC STUDIES OF THE TESTOSTERONE–AQUEOUS D-GLUCOSE INTERFACE

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Electro-osmosis and streaming-potential measurements were made across a testosterone-plug membrane, using water and aqueous solutions of D-glucose as permeants. The electrophoretic velocity of testosterone particles dispersed in these solutions was also measured, experiments being confined to the range where linear flux–force relationships hold. Phenomenological coefficients were evaluated by using these linear relations, and the results analyzed in the light of the thermodynamics of irreversible processes. Saxen's relationship holds between electro-osmosis and streaming potential. Concentration dependence of the various phenomenological coefficients was also examined. Cross-phenomenological coefficients were found to decrease with increase in the concentration of D-glucose solutions. The results are explained on the basis of strong hydrogen-bonding between D-glucose and the surrounding water molecules. Such membrane parameters as pore size, average number of pores, and the membrane constant were evaluated. Electro-osmotic and electrophoretic data were used to estimate the zeta potential, in order to characterize the membrane–permeant interface. The dependence of the zeta potential on the concentration was also examined.

INTRODUCTION

Lipids and proteins[1] constitute the most essential part of biological membranes, and carbohydrates are also found in biomembranes[2]. Carbohydrates attached to lipoproteins and situated at the outer surfaces of membranes act as binding sites for various chemical compounds. Biological membranes are structurally very complex, and hence, model membranes that are artificial analogs of natural membranes[3,4] have been studied in order that the transport behavior of natural membranes may be understood. It may be noted that these model membranes possess certain dimensional, electrical permeability, and excitability characteristics that mimic the behavior of biological membranes[4].

Electro-osmotic and electrophoretic transport studies on membranes of biologically important materials[5,6] have recently been made from the standpoint of the phenomenological theory of irreversible thermodynamics. Electro-osmosis, a nonequilibrium phenomenon, has been extensively investigated, because of the

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ease with which it can be measured and its various technological and biological applications. In earlier communications[6,7] we reported results of a physicochemical study of a testosterone interface indifferent types of electrolytic and non-electrolytic solutions of NaCl, KCl, $MgCl_2$, and urea.

Electrokinetic studies conducted with a testosterone-plug membrane, and using water and aqueous solutions of D-glucose, a compound of great physiological importance[8], are now reported. The membrane used in the present investigation was not a model membrane, but the results obtained with it may be helpful in understanding the electrical nature of the membrane–permeant interface. Such studies are important in view of the fact that surface activity and biological effects are closely related[9].

EXPERIMENTAL

Testosterone, obtained from Sigma Chemical Company (Saint Louis, U.S.A.), was used as such. D-Glucose (AR, BDH) and double-distilled water were used in these studies.

The method of preparation of the membrane plug and the electrodes, the experimental set-up, and the procedure for the measurement of various transport properties have already been reported[6]. Potential differences of up to 50 V were applied with the help of an electronically operated power-supply (Toshniwal and Company, India), using cylindrical, silver–silver chloride electrodes which pressed the membrane surface on both sides. Almost the entire membrane area was covered by the electrodes. The volumetric flux induced by the pressure difference and the potential difference was measured as described earlier[6]. The conductance of the membrane equilibrated with the permeant was measured with an A.C. conductivity bridge (Toshniwal, India) at 50 Hz, All measurements were made on systems in an air thermostat maintained at 30 $\pm$0.5°.

RESULTS AND DISCUSSION

According to nonequilibrium thermodynamics[10], the volumetric flux, J_v, and electric current, I, through a permeant-equilibrated membrane under the simultaneous action of a hydrodynamic pressure difference (ΔP) and an electric potential difference ($\Delta\phi$) are given by

$$J_v = L_{11}/T \cdot \Delta P + L_{12}/T \cdot \Delta\phi, \qquad (1)$$

and

$$I = L_{21}/T \cdot \Delta P + L_{22}/T \cdot \Delta\phi, \qquad (2)$$

where the parameters L_{ij} (i,j = 1,2) are called phenomenological coefficients.

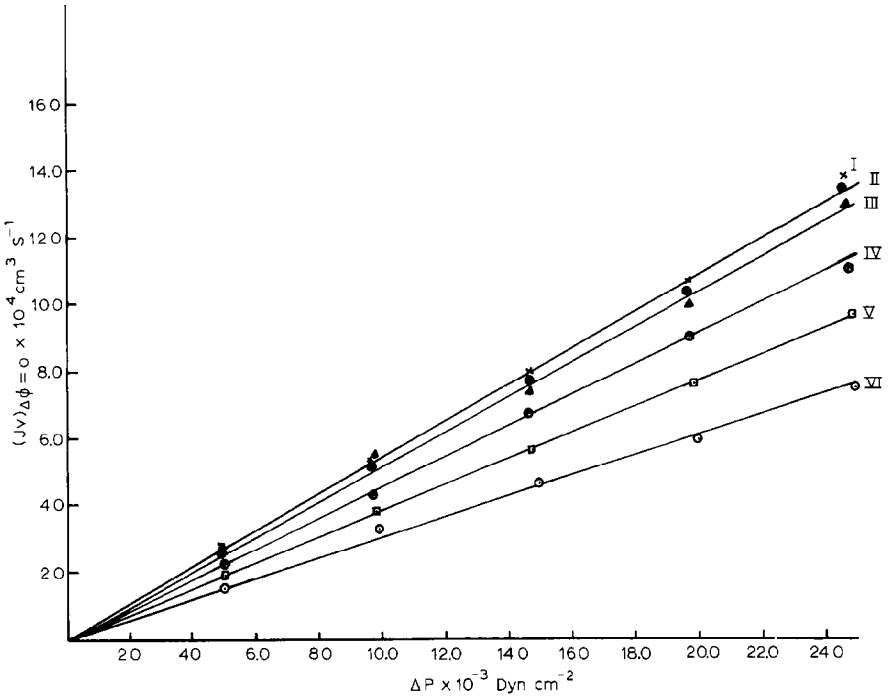


Fig. 1. Hydrodynamic permeability curve for the testosterone–D-glucose–water system. [Key: I, water; II, 0.1mM; III, 0.2mM; IV, 0.4mM; V, 0.6mM; VI, mM D-glucose.]

Eq. *1* predicts a linear dependence between (*i*) the hydrodynamic volume flux $(J_v)_{\Delta\phi=0}$ and ΔP, and (*ii*) the electro-osmotic volume flux $(J_v)_{\Delta P=0}$ and $\Delta\phi$, as shown in Figs. 1 and 2. The values of the phenomenological coefficients L_{11}/T and L_{12}/T, obtained from the slopes of Figs. 1 and 2, are recorded in Table I. Eq. *2* predicts a linear dependence between the streaming potential $(\Delta\phi)_{I=0}$ and ΔP, as shown in Fig. 3. Thus, from Eq. *2*,

$$-(\Delta\phi)_{I=0} = \frac{(L_{21}/T)}{(L_{22}/T)} \cdot \Delta P, \tag{3}$$

where L_{22}/T is defined as the conductance of the membrane equilibrated with the permeant. Its value was determined by measuring the conductance of the membrane–permeant system with a Toshniwal conductivity bridge. The product of the slopes $(L_{21}/T)/(L_{22}/T)$ and L_{22}/T yielded the value of L_{21}/T. The values of these phenomenological coefficients, recorded in Table I, validate Saxen's relationship within the limits of experimental error. It may be noted that $(\Delta\phi)_{I=0}$ was determined by measuring the potential differences at a particular pressure ΔP, and the potential difference at $\Delta P = 0$, and taking their difference.

TABLE I

PHENOMENOLOGICAL COEFFICIENTS AND MEMBRANE PARAMETERS FOR TESTOSTERONE–AQUEOUS D-GLUCOSE SYSTEMS

Conc. $\times 10^4$ (M)	$L_{22}/T \times 10^6$ *(mho)*	$L_{11}/T \times 10^8$ $(cm^5.s^{-1}.dyn^{-1})$	$L_{12}/T \times 10^6$ $(cm^3.s^{-1}.V^{-1})$	$L_{21}/T \times 10^6$ $(cm.s^{-1}.V^{-1})$	$L^*_{12}/T \times 10^6$ $(cm^3.s^{-1}.V^{-1})$	$\zeta_{e\,o}$ *(mV)*	$\zeta_{e\,p}$ *(mV)*	$n \times 10^{-5}$	$r \times 10^4$ *(cm)*	$K_m \times 10^2$ *(cm)*
0.0	4.50	5.63	2.75	2.73	3.30	5.75	5.86	1.964	2.526	5.62
1.0	4.54	5.27	2.58	2.58	3.27	5.22	5.80	2.243	2.405	5.82
2.0	4.60	5.06	2.32	2.27	3.06	4.60	5.48	2.209	2.455	5.97
4.0	4.65	4.56	2.04	2.20	2.83	4.37	5.20	2.389	2.300	5.67
6.0	4.72	3.98	1.66	1.58	2.70	3.52	5.00	2.816	2.139	5.78
10.0	4.80	3.65	1.34	1.40	2.44	2.86	4.70	3.162	2.056	6.00

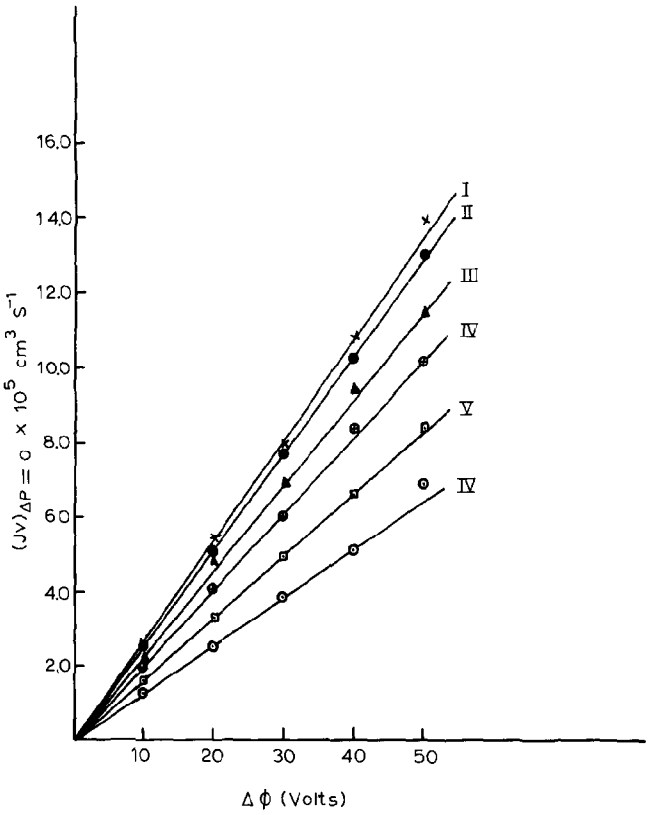


Fig. 2. Electro-osmotic permeability curve for the testosterone–D-glucose–water system. [Key: I, water; II, 0.1mM; III, 0.2mM; IV, 0.4mM; V, 0.6mM; VI, mM D-glucose.]

Electophoretic migration of testosterone particles dispersed in these solutions occurs towards the negative electrode, and the following linear equation satisfies the experimental data.

$$(V_e)_{g=0} = L_{12}^* \frac{\Delta\phi}{T} \quad , \tag{4}$$

where L_{12}^*/T, the electrophoretic transport coefficient, has the value

$$L_{12}^*/T = \frac{\epsilon\zeta_{e.p.}}{4\pi\eta\, l'} \quad , \tag{5}$$

ϵ and η being the dielectric constant and viscosity of the aqueous solutions of D-glucose in which the testoterone particles are dispersed to form a homogeneous suspension, $\zeta_{e.p.}$ is the zeta potential, and l' is the distance between the two electrodes of the elec-

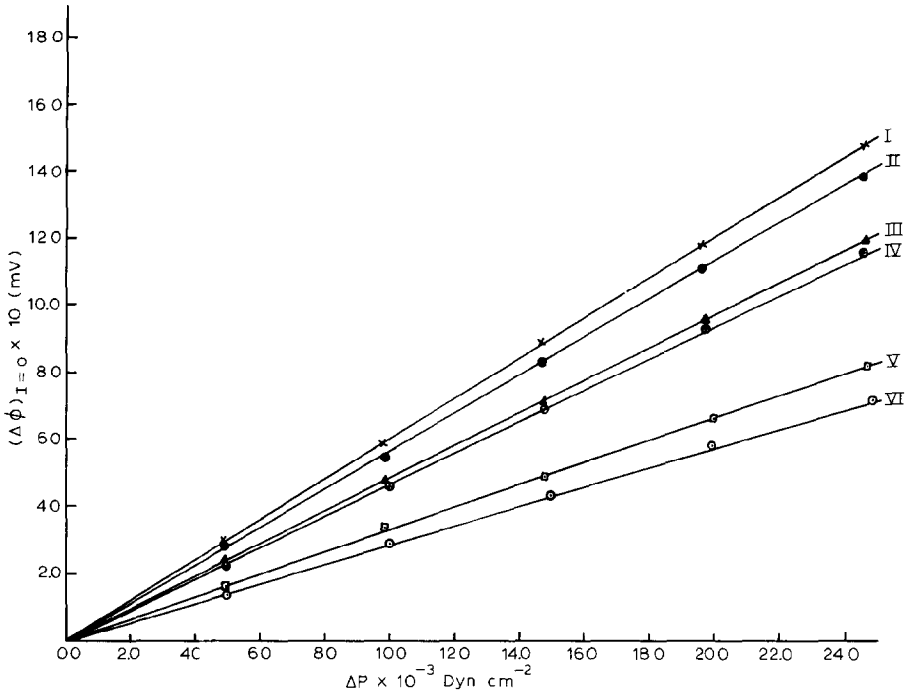


Fig. 3. Variation of streaming potential with pressure difference for the testosterone–D-glucose–water system. [Key: I, water; II, 0.1mM; III, 0.2mM; IV, 0.4mM; V, 0.6mM; VI, mM D-glucose.]

trophoretic cell. The values of L_{12}^{*}/T as obtained from the slopes of linear plots of Eq. *4* are recorded in Table I.

Estimation of membrane parameters. — The membrane was characterized by estimating the values of various parameters, *e.g.*, average pore-radius (r), average number of pores (n), and the membrane constant (K_m) by using the relations[6]

$$r = \left[\frac{8\eta\chi\, L_{11}/T}{L_{22}/T}\right]^{1/2}, \tag{6}$$

$$n = \frac{L_{22}/T \times l}{\pi r^2 \chi}, \tag{7}$$

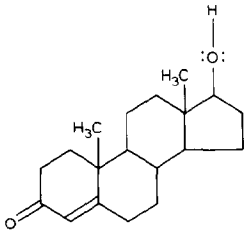
$$K_m = A_e/l = \frac{L_{22}/T}{\chi}. \tag{8}$$

The values of these parameters are recorded in Table I.

An examination of Table I reveals that the magnitude of L_{11}/T follows the trend $(L_{11}/T)_{water} > (L_{11}/T)_{D\text{-glucose}}$. This is attributable to the structure-making

property of D-glucose. It may be inferred that the structure-making tendency follows the order D-glucose > water.

Electro-osmotic flux occurs towards the anode. This may be explained on the basis of an electrical double-layer formed at the testosterone–solution interface. Testosterone has the following molecular structure.



The presence of (*i*) $>C^{\delta+}=O^{\delta-}$ at the α-carbon atom, (*ii*) π-electron density around the olefinic bond, and (*iii*) the oxygen atom of OH-5 suggests the possibility that these might be collectively responsible for generating an overall, negative charge at the testosterone molecule present on the matrix of the membrane. D-Glucose shows negative excess heat, entropy, free energy, and volumes[11], indicating that it is in a state of strong hydrogen-bonding with surrounding water molecules. The possible mechanism for the formation of a hydrogen bond is now outlined. The water molecule dissociates as follows.

$$H_2O + H_2O \rightleftharpoons H_3^+O + OH^-$$

The hydronium ion is adsorbed at the negatively charged, membrane surface, as shown in Fig. 4, and forms the I.H.P. The H^+ ions at I.H.P. form strong hydrogen-

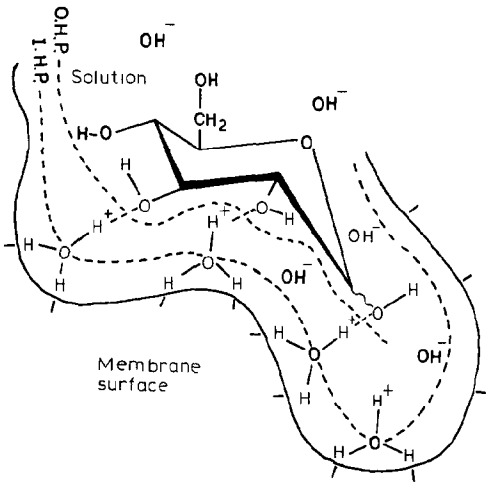


Fig. 4. Structure of the electrical double-layer at the interface of the testosterone–D-glucose solution.

bonds with the oxygen atoms of the hydroxyl groups present in the D-glucose molecule, forming the O.H.P. The OH^- ions are present in the bulk of the solution, and are also present in the diffused part of the electrical double-layer. Thus, electro-osmosis occurs from the negative to the positive electrode.

During electrophoresis, the testosterone particles are surrounded by the positively charged dipoles of the permeant, thus facilitating the migration of particles towards the cathode under the influence of the applied potential gradient, as observed experimentally.

Estimation of zeta potentials. — Electrokinetic effects are observed in porous membranes because of the formation of an electrical double-layer at the membrane–permeant interface. According to Overbeek[12],the rate of volume flux as a result of application of an electrical potential difference ($\Delta\phi$) and a pressure difference (ΔP) across the membrane is given by

$$(J_v)_{\Delta P=O} = \frac{n\varepsilon r^2 \cdot \zeta_{e.o.}}{4\eta l} \cdot \Delta\phi, \tag{9}$$

and

$$(J_v)_{\Delta\phi=O} = \frac{n\pi r^4}{8\eta l} \cdot \Delta P. \tag{10}$$

Within the range of the experimental investigation, comparison of Eqs. *9* and *10* with Eq. *1* gives

$$L_{12}/T = \frac{n\varepsilon r^2 \zeta_{e.o.}}{4\eta l} \tag{11}$$

and

$$L_{11}/T = \frac{n\pi r^4}{8\eta l} . \tag{12}$$

The value of $\zeta_{e.o.}$ can be estimated by combining the membrane conductance data and the electro-osmotic flux data. The conductance of the membrane equilibrated with the permeant may be expressed by Eq. *13*.

$$L_{22}/T = \frac{n\pi r^2}{l} \cdot \chi \tag{13}$$

Using Eqs. *11* and *13*, it is found that

$$\zeta_{e.o.} = \frac{4\pi\eta\chi}{\varepsilon (L_{22}/T)} \cdot (L_{12}/T) \text{ esu},$$

or

$$\zeta_{e.o} = \frac{4\pi\eta\chi}{\varepsilon\,(L_{22}/T)} \cdot (L_{12}/T) \times 90 \text{ kV}. \tag{14}$$

From electrophoretic-transport data, the zeta potential is given by following equation

$$\zeta_{e.p.} = \frac{4\pi\eta\, l'}{\varepsilon} \cdot (L^*_{12}/T) \text{ esu},$$

or

$$\zeta_{e.p.} = \frac{4\pi\eta\, l'}{\varepsilon} \cdot (L^*_{12}/T) \times 90 \text{ kV}. \tag{15}$$

The calculated values of zeta potentials using Eqs. *14* and *15* are given in Table I. It is obvious that the value of the zeta potential decreases at higher concentrations. This behavior can be explained on the basis of an altered structure of water that is likely to affect the electroosmotic and electrophoretic behavior. The dependence of the zeta potential on the concentration can be expressed by the following empirical equation[7] (see Fig. 5).

$$\zeta = A - B \log C, \tag{16}$$

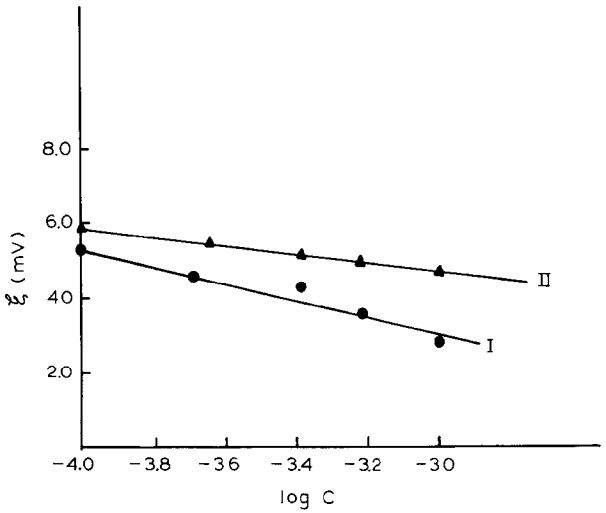


Fig. 5. Variation of the zeta potential with the concentration of the D-glucose solution. [I, From electroosmosis; II, from electrophoresis.]

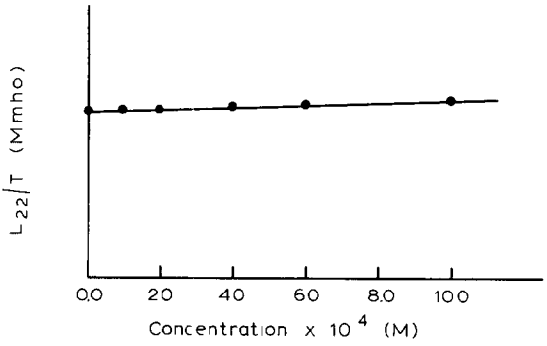


Fig. 6. Variation of L_{22}/T with the concentration of the D-glucose solution.

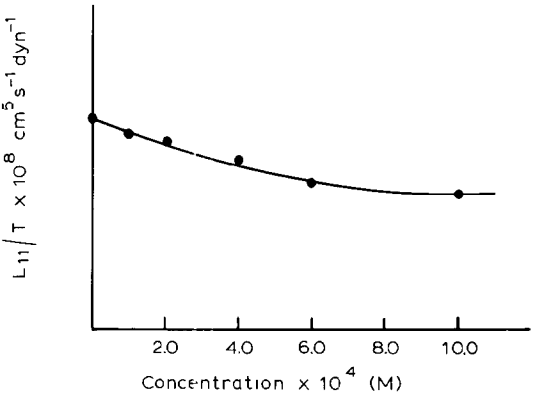


Fig. 7. Variation of L_{11}/T with the concentration of the D-glucose solution.

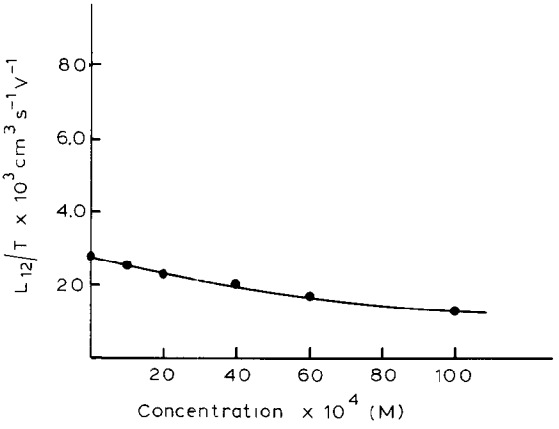


Fig. 8. Variation of L_{12}/T with the concentration of the D-glucose solution.

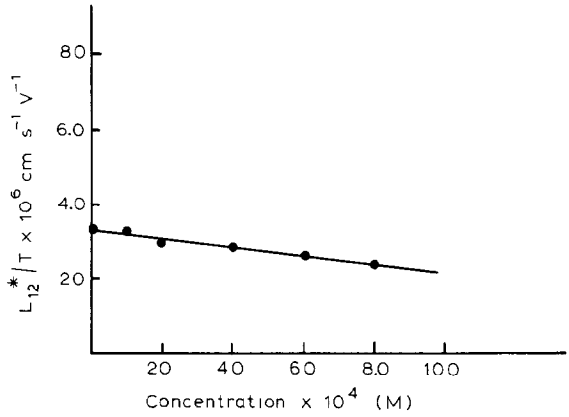


Fig. 9. Variation of L_{12}^*/T with the concentration of the D-glucose solution.

where A may be taken as an approximately constant quantity for conditions under which the charge on the membrane surface does not change sign and B is another constant.

Concentration dependence of phenomenological coefficients. — The dependence of phenomenological coefficients on the concentration of D-glucose solutions is shown in Figs. 6–9. The coefficients L_{11}, L_{12}, and L_{12}^* are found to decrease with the concentrations whereas L_{22} remains almost constant. The results may be explained with the help of Eqs. *5*, *11*, and *12*. According to these equations, it can be shown that

$$L_{11}/T \propto 1/\eta, \tag{17}$$

$$L_{12}/T \propto \frac{\zeta_{e.o.}}{\eta}, \tag{18}$$

and

$$L_{12}^*/T \propto \frac{\zeta_{e.o.}}{\eta}, \tag{19}$$

provided that n, π, ε, and l are constant.

At higher concentrations, the hydrogen bonding between D-glucose and surrounding water molecules becomes more and more pronounced, thereby increasing the viscosity. The decreasing values of ζ, and increasing values of η, at higher concentrations are such that ζ/η decreases appreciably. There is no appreciable change in the L_{22} values; this is mainly due to the fact that the specific conductivity remains almost unchanged in the range of concentration studied.

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